providing a first nucleic acid sample from each of said individuals;

providing a second nucleic acid sample by fragmenting said first nucleic acid sample to produce fragments, binding said fragments to a mismatch binding protein, and isolating said bound fragments;

providing a plurality of identical nucleic acid arrays wherein said arrays comprise probes which are designed to interrogate for DNA sequence variations;

hybridizing each of said second nucleic acid samples to one of said plurality of identical arrays; and

generating a plurality of hybridization patterns resulting from said hybridizations; and

analyzing the hybridization patterns to determine the presence or absence of sequence variation in the population of individuals.

173. (New) The method of claim 172 wherein said sequence variation is a single nucleotide polymorphism.

REMARKS

Please see Attachment A for marked up versions of the deleted paragraphs. Claims 1-37 have been canceled, and new claims 39-173 have been added. The canceled claims are included in Attachment B. Examination of the subject application, as amended, is respectfully requested.

Respectfully submitted,

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Attachment A: Marked up paragraphs according to 1.121(b)(iii):

Page 1, title:

Complexity [Management] Management and Analysis of Genomic DNA

Page 6, paragraph beginning on line 10:

An array comprises a solid support with nucleic acid probes attached to said support. Arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. These arrays, also described as "microarrays" or colloquially "chips" have been generally described in the art, for example, U.S. Pat. Nos. 5,143,854, 5445934, 5,744,305, 5,677,195 and PCT Patent Publication Nos. WO 90/15070 and 92/10092[. Each], each of which is incorporated by reference in its entirety for all purposes. These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase synthesis methods. See Fodor et al., Science, 251:767-777 (1991), Pirrung et al., U.S. Pat. No. 5,143,854 (see also PCT Application No. WO 90/15070) and Fodor et al., PCT Publication No. WO 92/10092 and U.S. Pat. No. 5,424,186, each of which is hereby incorporated in its entirety by reference for all purposes. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, e.g., U.S. Pat. No. 5,384,261, incorporated herein by reference in its entirety for all purposes. Although a planar array surface is preferred, the array may be fabricated on a surface of virtually any shape or even a multiplicity of surfaces. Arrays may be nucleic acids on beads, fibers such as fiber optics, glass or any other appropriate substrate, see US Patent Nos. 5,770,358, 5,789,162, 5,708,153 and 5,800,992 which are hereby incorporated in their entirety for all purposes. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of in an all inclusive device, see for example, US Patent Nos. 5,856,174 and 5,922,591 incorporated in their entirety by reference for all purposes.

Page 10-11, paragraph beginning on page 10, line 20:

After isolation, adaptor sequences are ligated to the fragments. (Figure 2, Step 4) Adaptor sequences are generally oligonucleotides of at least 5 or 10 bases and preferably no more than 50 or 60 bases in length, however, adaptor sequences may be even longer, up to 100 or 200 bases depending upon the desired result. For example, if the desired outcome is to prevent amplification of a particular fragment, longer adaptor sequences designed to form stem loops or other tertiary structures may be ligated to the fragment. Adaptor sequences may be synthesized using any methods known to those of skill in the art. For the purposes of this invention they may, as options, comprise templates for PCR primers and/or tag or recognition sequences. The design and use of tag sequences is described in US Patent No. 5,800,992 and US Provisional Patent Application No. 60/140,359, filed 6/23/99[. Both] ,both of which are incorporated by reference for all purposes. Adaptor sequences may be ligated to either blunt end or sticky end DNA. Methods of ligation will be known to those of skill in the art and are described, for example, in Sambrook et al. Methods include DNase digestion to "nick"

the DNA, ligation with ddNTP and the use of polymerase I to fill in gaps or any other methods described in the art.

Page 11, paragraph beginning on line 6:

Further complexity reduction is achieved by adding a specific nucleotide on the 3' [5'] end of the PCR primer as illustrated in Figure 3. The specific nucleotide further reduces the complexity of the resulting DNA pool because only those fragments which have been isolated after restriction enzyme digestion and contain the complement of the specific nucleotide(s) incorporated in the PCR primer will be amplified. Figure 3A depicts the results of hybridization to an array after enzyme digestion, ligation to an adaptor and PCR amplification. Figs. 3B and 3C depict the results of hybridization to an array after enzyme digestion, ligation to an adaptor and PCR amplification where the PCR primers incorporated specific nucleotides in the 3' [5'] end of the primer. In Fig. 3B the 5' and 3' primers have different specific nucleotides incorporated. In Fig. 3A the 5' and 3' primers have the same nucleotides incorporated. The level of complexity in the isolated pool can be varied depending upon the identity and number of nucleotides incorporated into the PCR primers. A number of embodiments of the present invention involve amplification by PCR. Any of these embodiments may be further modified to reduce complexity using the above disclosed technique.

Page 14-15, paragraph beginning on page 14, line 26

In another embodiment, the step of complexity management comprises performing an arbitrarily primed polymerase chain reaction (AP PCR) upon the sample. AP PCR is described in US Patent No. 5,487,985 which is hereby incorporated by reference in its entirety for all purposes. Figure 7 depicts a schematic illustration of this embodiment. Performing AP PCR with random primers which have specific nucleotides incorporated into the primers produces a reduced representation of genomic DNA in a reproducible manner. Figure 8 shows the level of complexity reduction of human genomic DNA resulting from AP PCR with various primers. Column 1 lists the primer name. Column 2 lists the primer sequence. Column 3 lists the annealing temperature. Column 4 lists the polymerase used. Column 5 lists the number correlated to a specific gene on the Hum6.8K GeneChip(R) probe array (Affymetrix, Inc. Santa Clara, Ca). Column 6 lists the percentage of the human genes on the Hum6.8K GeneChip(R) probe array found by fragments whose complexity has been reduced by this method. Figure 9 shows the reproducibility of AP PCR. Independently prepared samples [preps] were subjected to AP PCR using the same primers. The gel bands show that the level of reproducibility between the samples is very high.

Page 25-26, paragraph beginning on page 25, line 25:

The labeled DNA was then hybridized to an array in a hybridization mixture containing 80 ug labeled DNA, 160 ug human COT-1 DNA (GIBCO), 3.5 M tetramethylamonium cloride, 10 mM MES (pH 6.5), 0.01% Triton-100, 20 ug herring sperm DNA, 100 ug bovine serum albumin and 200 pM control oligomer at 44 °C for 40 hours on a rotisserie at 40 rpm. The arrays were then washed with 0.1 M MaCl in 10 mM MES at 44 °C for 30 minutes on a rotisserie at 40 rpm. The hybridized arrays were then stained with a staining solution [10 mM MES (ph 6.5), 1 M NaCl, 10 ug/ml steptaviden R-phycoerythrin, 0.5 mg/ml acetylated BSA, 0.01% Triton-100] at 40 °C for 15 minutes. The arrays were then washed with 6x SSPET [0.9 M NaCl, 60 mM NaH2PO4 (pH 7.4), 6 mM EDTA, 0.005 % Triton-100] on a GeneChip® Fluidics Station (Affymetrix, Inc., Santa Clara, CA) 10 times at 22 °C. The arrays were then anti-streptavidin antibody stained at 40 °C for 30 minutes with antibody solution [10mM MES (pH 6.5), 1 M NaCl, 10 ug/ml streptavidin R-phycoerythrin, 0.5 mg/ml actylated BSA, 0.01% Triton-100]. The arrays [are] were then restained with staining solution for 15 minutes followed by 6X SSPET washing as above. The arrays [are] were then scanned with a confocal scanner at 560 nm. The hybridization patterns were then screened for SNP detection with a computer program as described in D.G. Wang et al Science 280, 1077-1082, 1998. The results of the hybridization can be seen in Figures 8A and 8B.

Page 30, paragraph beginning on line 24:

PCR was performed in a MJ Research Tetrad thermocycler using an initial 10 minute denaturation at 95 °C, 35 cycles of a 0.5 minute denaturation at 94 °C, annealing for 0.5 minute at 57 °C and extension at 72 °C. This [is] was followed by a final 5 minute extension at 72 °C.

Page 31, paragraph beginning on line 7:

The sample was then denatured at 95 °C for 10 minutes and allowed to reanneal by slowly cooling to room temperature.

Page 32, paragraph beginning on line 8:

PCR was performed in a MJ Research Tetrad Thermocycler using an initial 10 minute denaturation at 95 °C, 45 cycles of a 0.5 minute denaturation at 94 °C, annealing for 0.5 minute at 52 °C and extension at 72 °C for 1 minute. This [is] was followed by a final 5 minute extension at 72 °C. The fragments were then labeled and hybridized to an array.

Page 33-34, paragraph beginning on page 33, line 28:

Correlation is performed for a population of individuals who have been tested for the presence or absence of a phenotypic trait of interest and for polymorphic markers sets. To perform such analysis, the presence or absence of a set of polymorphisms (i.e. a polymorphic set) is determined for a set of the individuals, some of whom exhibit a particular trait, and some of which exhibit lack of the trait. The alleles of each

polymorphism of the set are then reviewed to determine whether the presence or absence of a particular allele is associated with the trait of interest. Correlation can be performed by standard statistical methods such as a [6-squared] chi-squared test and statistically significant correlations between polymorphic form(s) and phenotypic characteristics are noted. For example, it might be found that the presence of allele A1 at polymorphism A correlates with heart disease. As a further example, it might be found that the combined presence of allele A1 at polymorphism A and allele B1 at polymorphism B correlates with increased milk production of a farm animal. (See, Beitz et al., US 5,292,639).

Page 36, paragraph beginning on line 24:

Similarly, some medications may be highly effective for only a very small percentage of the population while proving only slightly effective or even ineffective to a large percentage of patients. Pharmacogenomics allows pharamaceutical companies to predict which patients would be the ideal [candidate] candidates for a particular drug, thereby dramatically reducing failure rates and providing greater incentive to companies to continue to conduct research into those drugs.